ACADEMIC PRESS

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Experimental Cell Research 285 (2003) 131–145

Experimental Cell Research

www.elsevier.com/locate/yexcr

Id proteins in epithelial cells

Jean-Philippe Coppé, Andrew P. Smith, and Pierre-Yves Desprez*

California Pacific Medical Center, Cancer Research Institute, San Francisco, CA 94115, USA

Received 2 August 2002, revised version received 18 November 2002

Abstract

Id helix-loop-helix (Id HLH) proteins are negative regulators of basic HLH transcription factors. They are expressed during embryonic development and are important for the regulation of cell phenotypes in adults. They participate in the molecular networks controlling cell growth, differentiation, and carcinogenesis, through specific basic HLH and non-basic HLH protein interactions. Recent in vitro and in vivo data implicate Id HLH as important orchestrating proteins of homeostasis in glandular and protective epithelia. In particular, Id proteins have been reported to be involved in cell behavior in epidermis, respiratory system, digestive tract, pancreas, liver, thyroid, urinary system, prostate, testis, endometrium, cervix, ovary, and mammary gland. The purpose of this review is to summarize the evidence implicating Id proteins in the regulation of mammalian epithelial cell phenotypes.

Introduction

Helix-loop-helix (HLH) transcription factors [1] are important players in the transcriptional network regulating cell growth and differentiation during many essential developmental processes, in both vertebrates and invertebrates (for reviews, see [2–4]). This family of protein comprises more than 240 members [3,5] that coordinate cell type-specific gene expression implicated in cell lineage determination, cell proliferation, and cell death and, globally, differentiation of most tissues [3,6–17]. They are commonly grouped into seven classes (I–VII), based on tissue distribution, dimerization capabilities, and DNA binding specificities [3,18].

A characteristic feature of HLH proteins essential to their normal function is the ability to form homo- and heterodimers through their highly conserved HLH domain (for reviews, see [3,13,18]). Most of these proteins belong to the basic helix-loop-helix (bHLH) family and act as transcriptional enhancers or inhibitors of various genes through direct DNA binding to the canonical E-box sequence [19]. However, the four members of the HLH class V subfamily [20,21], known as Id proteins (*i*nhibitor of *D*NA binding) act as dominant negative regulators of transcription factors. While they contain the HLH domain enabling them to dimerize with other members of the family (classes I and II) [3], the resulting Id-bHLH heterodimers are unable to bind to DNA because Id HLHs lack the basic motif necessary for this [20].

The four Id proteins differ substantially with respect to their expression patterns during mouse embryogenesis [11]. During mouse gastrulation, Id4 is absent while Id1, 2, and 3 exhibit specific and distinct patterns of expression. During neurogenesis, each of the four Id members is present in a unique expression pattern, which persists over neuronal development. During murine postgastrulation, Id1-2-3 are expressed in multiple tissues whereas Id4 is restricted to the ventral portion of the developing stomach epithelium and in neuronal tissues. Id1-2-3 are expressed in tissues undergoing active morphogenic activities, and overlap in many tissues but not in primitive gut-derived tissues. Later, Id2 is the only member of this family to be expressed within the epithelium, while Id1 and Id3 are present in mesenchyme surrounding the epithelium. The varying levels and overlapping patterns of expression of Id proteins contribute to the specific differentiation process during development.

As with many other essential proteins, strong evidence

* Corresponding author. California Pacific Medical Center, Cancer Research Institute, 2330 Clay Street, Stern Building, San Francisco, CA 94115, USA. Fax: +1-415-561-1390.

E-mail address: pdesprez@cooper.cpmc.org (P.-Y. Desprez).

0014-4827/03/$ – see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S0014-4827(03)00014-4

now suggests that Ids are key regulators of oncogenic transformation and/or other cellular processes related to the growth of malignancies [8,10,13,14,16,17,22,23]. Ids are generally overexpressed in tumor cells, their presence usually, though not always, correlating with that of dedifferentiated, proliferative, and invasive cell phenotypes. Moreover, before metastasizing and invading tissues beyond their point of origin, most cancerous cells emerge from epithelia that are themselves undergoing advanced tumorigenic processes. Epithelia are relatively avascular, mainly consisting of cells with almost no connective tissue, and specialized for absorptive, secretory, protective, or sensory activities. They may be distinguished between those covering and lining epithelium, and those with secretory or glandular functions. The control of mammalian epithelial cell phenotype is one of the major functions of Ids, from normal differentiation and proliferation processes to the mechanisms of cancer development.

The purpose of this review is to summarize and interpret the evidence implicating Id proteins in the regulation of epithelial cell phenotypes, occurring in normal differentiation as well as tumorigenesis. These data are organized in a functional organotypic presentation, and summarized in Tables 1 and 2. Molecular pathways regulating epithelial phenotypes through Ids are discussed as well as the study of the function of bHLH and certain non-HLH proteins in epithelial cells and their potential relevance to Ids. This review will be limited to mammalian epithelium, mainly human and mouse, and will not cover studies of epithelial tissues lining closed internal body cavities such as endothelium (blood vessels) and mesothelium (serous cavities), nor the tissues of the nervous system, which originate from ectoderm. The roles of Id proteins in these tissues have been extensively reviewed by others [2,6–8]. We will be concerned only with differentiation processes occurring in the adult, as illuminated by in vitro and in vivo studies, as well as tumorigenesis, and not with development or embryogenesis.

Epidermis

The epidermis undergoes complex dynamic processes [24] in which keratinocytes migrate and differentiate from the proliferating basal immature cell layers to the superficial squamous epidermal layers. Id1-2-3 are downregulated in differentiating keratinocytes cultured in vitro, and within in vivo skin sections and living skin equivalent models [25,26]. After differentiation is completed, however, Id expression is undetectable. In contrast, immunostaining of the proliferating basal layers of the epidermis is able to detect Ids, including cytoplasmic Id1 and nuclear Id2-3 [26]. In situ hybridization (ISH) localizes Id1 within basal and spinous layers. Some columns of Id2-3-expressing cells are also observed in potential epidermal proliferative units.

In vitro experiments using human primary keratinocytes and normal human epidermal keratinocytes (NHEK cells), and mimicking cell-cell and cell-matrix loss of contact, display upregulation of Id1 during keratinocyte dedifferentiation [25,26]. Consistent with this, in vitro ectopic expression of Id1 in primary human foreskin keratinocytes (HFK cells) reduces their differentiation capacity [27]. Id1 may also participate in epidermal wound healing processes. Id1 can facilitate the phenotype transition from resting, sessile keratinocytes to mobile, migrating, and proliferating keratinocytes during the repair of epidermal lesions and reepithelialization [25].

Cell lines derived from cancerous squamous epithelia and incapable of differentiation (squamous cell carcinoma SCC9, and HaCaT) highly express Id1-2-3 mRNAs [25,26]. Moreover, sections from well-differentiated SCC display a lack of Id immunoreactivity, whereas the most poorly differentiated and malignant biopsy sections of squamous cell carcinoma show high cytoplasmic Id1 immunoreactivity and abundant levels of Id3—and to a lesser extent Id2—within the nucleus [26]. High levels of Id1 expression are also found in the cell cytoplasm of lesional epidermis areas of bullous pemphigoid [25]. Finally, the level of Id expression correlates directly with disease course and the propensity to metastasize [26]. Taken together, these data suggest that a high or upregulated level of expression of Ids is related to an undifferentiated, proliferative keratinocyte phenotype, found during normal as well as cancerous processes.

However, the effects of Ids on cell lifespan and death of keratinocytes (HFK cells) are the subject of some disagreement. Alani et al. [27] reported that Id1-2-3 were all able to extend the normal primary human keratinocyte lifespan. The ectopic expression of Id1 alone was sufficient to immortalize primary human keratinocytes, previously transfected with and selected for an Id1 expression vector [27]. This was accompanied by a constellation of molecular changes, including reduced differentiation capacity, pRb inactivation through phosphorylation inactivation of the pRb tumor-suppressor pathway, and the cyclinD-CDK4/6-p16 pathway, increased telomerase activity, and impairment of the p53-mediated DNA damage response during the late immortalization stages [27]. This analysis is in agreement with a previously reported description of a genetic program activated during the keratinocyte immortalization process [28].

However, this conclusion contrasts with that of a more recent report [29] using a retroviral Id1 expression system and unselected human keratinocyte populations. Senescence was clearly delayed by Id1 overexpression but was not prevented, and immortalization did not occur. No increases in telomerase activity or in p16 and active Rb levels were observed. Still another study using an HaCaT squamous cell line overexpressing Id1 or Id2 or Id3 [26] failed to produce an organotypic model, suggesting a potential role of Ids in promoting keratinocyte death, and perhaps apoptosis under certain circumstances.

Table 1
Expression of Id proteins in protective and glandular epithelia[a]

Tissue [references]	Observed tissue/cell phenotype	Id	Expression (method)	Modified Id expression	Related phenotype
Epidermis [25–27, 29]	During differentiation	Id-1,2,3	↘ (ISH, IHC)	↗ Id-1 in normal keratinocytes	Reduced differentiation capacity and extended lifespan
	Differentiated cells	Id-1,2,3	−(ISH, IHC)		
	Normal proliferation	Id-1,2,3	++(ISH, IHC)	↗ Id-1 in primary keratinocytes	Immortalization, or delayed senescence, or cell death (?)
	Tumors, bullous pemphigoid	Id-1,2,3	+++ (ISH, IHC, cc)		
Lung [42–44]	During differentiation	Id-1,2	↗ (ISH, IHC)		
	Tumor cells	Id-1,2	++?(cc)		
Esophagous, oropharynx [26,46]	Differentiated cells	Id-1,2	−(IHC)		
	Tumor cells	Id-1	+++ (IHC, cc)		
Small intestine [52,53]	Normal differentiating and migratory cells	Id-1	=(IHC)	Id1−/− mice	No effect
		Id-2,3	↗ (IHC)	↗Id-1 in murine intestine	Adenomas; no carcinomas; Id-2,3 ↘
Colorectal intestine [44,48,51,53]	Normal differentiated	Id-1,2,3	+ (IHC)	↗ Id-2 in cell lines	Increased proliferation and malignancy
	Premalignant adenomas	Id-1,2,3	++ (IHC, cc)		
	Tumors, adenomatous polyps	Id-1,2,3	+++ (IHC, cc)		
Pancreas [65–67,78,79]	Maturing cells	Id-1,2	↗(cc)	↗Id-1,2 in cell lines	Cell growth activation, and inhibition of differentiation
	Tumor cells	Id-1,2,3	+++ (ISH, IHC, cc)		
				↘Id-2 in cell lines	Cell growth inhibition
Liver [80,81]	During differentiation	Id-1	↘ (ISH, cc)		
	Differentiated cells	Id-1	− (ISH, cc)		
	Normal proliferation	Id-1	++ (ISH, cc)		
Thyroid [84]	Induced cell proliferation	Id-1	↗ (cc)	↗Id-1 in cell lines	Further increase of cell growth
	During differentiation	Id-1	↘ (cc)		
	Tumor cells	Id-1	+++ (IHC, cc)		
Kidney [87–89]	Differentiated cells	Id-4	++		
Prostate [101–103,107, 111,112]	Premalignant tumors	Id-1	+ (cDNA array)	↘ Id-1 in cell lines	Reduction of proliferation
	Malignant invasive tumors	Id-1	++ ? (cDNA array, IHC, ISH, cc)		
Sertoli cells [113–115]	Mature nondividing cells	Id-2,3	++		
		Id-1,4	−		
Endometrium [120]	Differentiated cells	Id-1	− (IHC)		
	Invasive tumors	Id-1	+++ (IHC)		
Cervix [121]	Tumors	Id-1	+++ (IHC)		
Ovary [122,123]	During tumor progression	Id-3	↘ (cDNA array)	↗Id-4 in cell lines	Increased malignancy
		Id-4	↗ (ribozyme lib)		
Mammary gland [123,125,126, 128–134,137]	During differentiation	Id-1	↘ (IHC, cc)	↗ Id-1 in normal or nonaggressive cell lines	Proliferative, invasive and migratory cells
		Id-2	↗ (IHC, cc)		
	Normal proliferating cells	Id-1	+++ (IHC, cc)	↗ Id-2 in cell lines Id2−/− mice	Increased differentiation
		Id-2	+ (IHC, cc)		Differentiation deffects
	Nonaggressive tumors	Id-1	+ (IHC)		
	Invasive tumors	Id-1	+++ (IHC, cc)		
		Id-2	+ (IHC, cc)		
		Id-4	+++ (ribozyme lib)		

[a] The observed endogenous or exogenously modified upregulation (↗) or downregulation (↘) of Id proteins is specified. Levels of Ids expression (stable: = ; absent or undetectable: −; low or detectable: +; high: ++; very high: +++; unclear data: ?) are indicated. The abbreviations used for the experimental methods: IHC, immunohistochemistry; ISH, in situ hybridization; cc, cell culture; lib, library.

Despite their inconsistency, these results do add to the evidence that Ids, particularly Id1 and also Id2, play a major role in the regulation of cell growth (cell cycle and cell death/senescence). These intricate but opposite roles of Ids controlling cell growth and death are typical of tumor regulator genes, and add to the potential characterization of Ids as oncogenes. The nature of these effects may be dependent on cell culture conditions (reviewed in [16,17]). As will be discussed later, data from a few other tissues suggest that different Ids can play opposite roles in the same tissue, while the same Id may play opposite roles in different tissues.

The molecular pathways through which Ids act in epidermis are not clear. So far, the only bHLHs that are well identified in epidermis and have been shown to dimerize and mediate signal transduction are Arnt and the dioxin receptor (in its ligand-activated state mediated through the tyrosine kinase activation pathway) [30], which do not interact with Ids. Also expressed, as in the majority of epithelial tissues, are the bHLH class I *E2A* gene products (E12

Table 2
Expression of Id proteins in various epithelial cancers[a]

	Epithelial cancer											
	Epidermis	Lung	Esophagous, oropharynx	Small intestine	Colorectal intestine	Pancreas	Thyroiod	Prostate	Endometrium	Cervix	Ovary	Mammary gland
Id-1	+ (G, I)	+	+ (G)	+	+	+ (G)	+ (G, I)	+ (G)	+	+		+ (G, I)
Id-2	+ (G, I)	+		−	+ (G)	+ (G)						−
Id-3	+ (I)			−	+	+					−	
Id-4											+ (I)	+ (I)
Ids-related molecular pathways			APC/β catenin-p16 Cyclin D1		β-catenin/TCF c-Myc p53 E47		RET	TGF-β p16/pRb			BRCA-1	BRCA-1 PgR NF-I TGF-β ITF-2

[a] The presence (+) or absence (−) of each of the four Id proteins (Id-1 to 4) is indicated as well as the cellular function of Ids when characterized (G, cell growth; I, cell invasion). Major molecular pathways related to Ids are indicated when relevant. Epithelia from the stomach, salivary gland, liver, spleen, kidney, bladder, uretrea, hair follicule, and cornea, as well as auditory and olfactory epithelia, have not been studied specifically, though we discuss some of them in the text as potential Id-regulated tissues.

and E47), which are present in the nucleus of keratinocytes throughout the whole epidermis [26]. Moreover, expression of the cyclin-dependent kinase inhibitor (CDKi) $p21^{cip1waf1}$ is inversely correlated with Id expression and increases over keratinocyte differentiation in vitro [26]. Id-2, and possibly Id-1, are able to interact with and inhibit Ets-2 activity or/and bHLH dimers, thus inhibiting potentially the SRE sites, and most probably the E-box sites within the *p21* promoter [31–35].

Finally, maintenance and (patho-) physiology of the epidermis are mainly under control of the dermis, e.g., through cytokine and interleukin signaling. Some evidence implicates EGF-R/EGF and IGF-IR/IGF-I pathways as positive regulators of Id1-2-3 expression in keratinocytes [26], as already described in other tissues for Id gene regulation [36–41]. Further investigations are needed to determine the role of Id genes in regulation of epidermis homeostasis.

Respiratory system

From the respiratory conducts to the lobar bronchus and the alveolar ducts, the mammalian airway epithelium is composed of many different cell populations that line all the divisions of the respiratory tract, including the pulmonary neuroendocrine cells. Expression patterns of Id1-2 have been analyzed in several lung cell lines and primary cultures of alveolar epithelial cells, comparing their profiles in proliferating fetal to nonproliferating adult cells. Id1-2 expression increases during the differentiation process, reaching a peak in adult alveolar cells supporting the highly differentiated nonproliferative phenotype. This suggests that these proteins act as positive regulators of alveolar epithelial cell differentiation [42], a conclusion consistent with previous studies implicating Id2 during lung morphogenesis and differentiation [43], though opposite from their role in epidermis, as discussed above.

Further studies of the role of Id2 suggest the existence of an homodimerization mechanism associated with inhibition of cyclin A promoter activity in lung epithelial-alveolar cells (A549 cell line). This Id2 homodimerization process occurs in vitro, and its functionality depends on (i) the HLH domain, (ii) a cysteine residue (Cys42 in the first of the characteristic two helices), and (iii) the HLH flanking region (a region extending over 25–100 amino acids). The role of Id1 and its potential HLH partner(s) remains to be elucidated.

Finally, in human lung cancer cell lines HUT69C and H292, very high levels of expression of Id1 mRNA and relatively high Id2 mRNA levels are observed [44]. However, no comparative data of Id expression in normal versus cancerous lung tissue are yet available.

Digestive system

Oropharyngial cavity and esophagous

Like the epidermis of the skin, the mucosal surface of the anterior end of the digestive tract, including the oral cavity, oropharynx, larynx, and esophagous, is a stratified squamous epithelium [45]. Cells from head and neck squamous cell cancer have been reported to express high levels of Id1, particularly the most undifferentiated proliferating cells ([26]; see above). A cDNA array-based study of esophageal squamous cell carcinoma (SCC) reported Id1 upregulation in derived cell lines of esophageal squamous cell carcinoma (HKESC-1 and HKESC-2) compared to morphologically

normal esophageal cells [46]. Furthermore, tissue immunohistochemistry showed a cytoplasmic Id1 overexpression within tumors while Id1 was almost undetectable in morphologically normal esophageal epithelium [46].

No bHLH class I or II proteins have yet been identified in cell growth control and transformation of this part of the digestive tract. However, the APC/β-catenin pathway may be involved in esophageal cancer [47], perhaps through the regulation of Id expression as has been suggested in colorectal cancer ([48]; see below). It is also interesting that altered p16 and cyclin D1 profiles of expression are associated with esophageal SCC [46,49], potentially linking Id expression and genes regulating cell cycle progression, as has been previously demonstrated in other tissues [13,14,16,17,23]. Finally, oral SCC seems to be related to p53 mutations but also manifests independently through p21 expression and transforming growth factor-beta (TGF-β) regulation [50], which have also been associated with Id dysregulation in colorectal cancer [51].

Small intestine

The intestinal epithelium is composed of four distinct cell types: three kinds of secretory cells, including goblet cells, enteroendocrine cells, and Paneth cells, and the enterocytes, which are absorptive cells. They all derive from multipotent stem cells. In the normal adult mouse small intestinal epithelium, a complex pattern of expression of Id1-2-3 exists [52]. Id2-3 are detectable in the nuclei of nondividing cells and increase during the differentiation-migration process occurring in each crypt-villus unit. In contrast, Id1 levels do not change during this period, and the protein remains restricted to the cellular cytoplasm. *E2A* gene products also localize to the intestinal epithelial cell cytoplasm, which suggests a potential role for E12/E47 in mediating the Id1-restricted localization pattern [52].

Previous findings have shown a limited contribution of Ids in establishing and regulating the homeostasis of the intestinal epithelium, since no particular histological intestinal phenotype has been associated with Id1−/− or Id3−/− mice [53]. Based on studies of chimeric mice [52], forced expression of Id1 in the intestinal epithelium does not seem to affect cell fate, nor modify Id localization. However, overexpressed Id1 proteins accumulate in the cytoplasm and are related to a drastic reduction of Id2-3 level of expression. Chimeric mice overexpressing Id1 in the small intestine epithelium exhibit a phenotype similar to that of mice carrying an *APC* mutation ([54]; and see below), with 15% of the animals developing adenomas but not carcinomas. No quantitative or qualitative changes in cell proliferation, lineage, differentiation, and migration are detectable. Id1 dysregulation alone may therefore not be sufficient to initiate small intestinal carcinogenesis.

In summary, homeostasis of the small intestine epithelium is not significantly affected by high/low levels of Id1-2-3, also suggesting a role of other factors in the initiation of intestinal tumorigenesis. However, some bHLH class II proteins are essential to intestinal cell commitment, differentiation, and function: BETA2/NeuroD [55,56], MATH-1, and Hes [57], which also links the Notch pathway to the bHLH network, and potentially Ids.

Colorectal intestine

In the human colonic epithelium, Id levels and patterns of expression are broadly similar to those previously described for small intestinal villosities and crypts [51]. Predominantly, Id1-2 have a cytoplasmic location, while Id3 is nuclear in human colorectal musoca [51]. However, one analysis failed to detect any Id2 expression in normal colon mucosa [48]. In the human colon carcinoma cell line Colo205 but not Colo320, overexpression of only Id1 mRNA was reported [44]. In human adenomatous polyps and colorectal tumor tissues, immunostaining clearly revealed higher levels of Id1-2-3 proteins compared to those of the normal colonic mucosa [48,51,53]. Id3 overexpression was also associated with a partial abnormal cytoplasmic localization. More interestingly, colorectal tumor tissues expressed higher levels of the Id1-2-3 proteins compared to premalignant adenomas [51]. That Id2 dysregulation is a major contributor to inappropriate cell growth is further suggested by the observation in other types of tissues, such as neuroblastoma, that Id2 level of expression is correlated with the rate of proliferation of primary, immortalized, and tumor cells [58,59]. Id2 overexpression also increases anchorage-independent survival of human colon carcinoma cells [48], while abolition of Id functionality leads to growth arrest of colorectal adenocarcinoma cell lines HCLO and LS147T [51].

Ids have been implicated in several possible molecular pathways contributing to colon carcinogenesis. The great majority of colon cancers are initiated by mutations in the *adenomatous polypsis coli* (APC) gene and/or *β-catenin* gene, in a process that also involves alterations in regulation of expression of the *c-myc* and *cyclin D1* genes [60]. These adenomas present a strikingly similar pattern of Id2 and β-catenin expression [48], with the increase of Id2 expression directly correlated with hyperactivity of the β-catenin/TCF pathway in colonic polyps. Id levels of expression are also well correlated with the mitotic index of human colorectal tumors and with p53 expression. The normal mucosa of p53-null mice expresses higher levels of Ids [51].

The Id2 promoter contains a functional TCF binding site activated by TCF-4, as observed in HT29 cells [48]. In colon cells, transformed or not, Id2 is a direct target of this signaling pathway, and its deregulation leads to the subsequent abnormal expression of Id2, and to a tumorigenic process. However, since the Id2 promoter contains myc-binding sites [58], its overexpression may also be induced by the protooncogene *c-myc*, which in turn is induced by the β-catenin/TCF pathway [61]. In fact, the myc-promoting effect on Id2 is required to bypass proliferation control by

Rb, a tumor suppressor protein. Rb sequestration by Id2 (and potentially Id4, but not Id1 or Id3) [14,58,62,63] would lead to uncontrolled cell growth. In HCLO and LS147T cells, overexpression of the E protein E47 (*E2A* product) initiates a reduced cell proliferation state, likely through Id1-2-3 inhibition/sequestration [51]. This would lead to unregulated bHLH activity and abolish E2A-mediated suppression of cell growth through unregulated p21/p16/p15 expression [31,64].

In any case, the main effect of Id2 overexpression is to allow unrestricted proliferation. The contribution of Ids to uncontrolled cell proliferation suggests that their major effects occur in relatively late phases of tumorigenesis in human colorectal epithelium.

Pancreas

Pancreatic normal phenotype and function

The pancreas is an endocrine gland, presenting a secretory multicellular epithelial structure, with both exocrine functions—a duct emptying into the lumen of another organ, and endocrine functions—ductless, secreting directly into blood and extracellular fluid, and producing hormones. The exocrine, endocrine, and ductal cells of the pancreas are highly specialized epithelial cells. Id1 and Id2 protein levels increase during the maturation-like process of β-cells into δ-cells, inversely correlating with insulin gene activation [65], and forced Id expression inhibits insulin production by insulinoma cells [66,67]. Ids may act by both inhibiting the *cis*-regulated insulin control element (ICE), and by sequestering E2A products that couple with HLH complexes and INSAF (insulin activator factor) to *trans*-regulate the insulin gene [67].

In addition, essential bHLH factors are well established as key regulators of pancreatic cell function and differentiation, e.g., NeuroD/BETA2, E47, Neurogenin3, HES-1, and PTF1-p48 as well as some of the non-bHLH Pax proteins [68–74]. In the pancreas, it is clear that Ids and bHLHs are part of a much larger protein network regulating the various functions of different types of cells. The Id2 gene is itself regulated by the type 1 insulin-like growth factor receptor (IGF-IR) and the insulin receptor substrate-1 (IRS-1) in murine hematopoietic cell lines [36,37]. The MAPK (mitogen-activated protein kinase) and PI3K (phopshatidylinositide 3-kinase) pathways as well as the Stat3 pathway also modulate Id2 expression (up/down regulation) [36].

Some studies have begun to delineate the role of Ids in this network of protein regulators. In the HIT pancreatic cell line, in which endogenous E2A-like activity is high, E2A and Id proteins regulate expression of the cyclin-dependent kinase inhibitor p21 (CDKi) [31]. p21 is overexpressed early during pancreatic intraepithelial neoplasia [75], and binds to cyclin-CDK complexes, resulting in the inhibition of kinase activity, and halting cell cycle progression. Id1 overexpression leads to cell growth activation and inhibits p21 expression [31,76]. Id1 may thereby be required during oncogenesis as part of the control of the cell cycle, at least in pancreatic cells and some other tissues where this network has been described (for reviews: [13,14,16,17,23].

Pancreatic carcinoma

Human pancreatic cancer is highly malignant and almost always fatal [77]. This cancer is often associated with oncogenic gene dysregulation and with overexpression of mitogenic growth factors and receptors. Ids have also been implicated in this tumorigenic process. An increase of Id1-2-3 mRNA levels (5-fold increase for Id1-2 and 2-fold for Id3) as well as protein levels is observed in pancreatic cancer tissues, and in ductal-like areas of chronic pancreatitis (CP) and dysplastic lesions of CP [78,79]. In contrast, normal pancreatic tissues express low to moderate levels of Id1-2 mRNA, though relatively high levels of Id3 mRNA [78,79]. Immunohistochemistry reveals that all three Ids colocalize in tumor cells and duct-like cancer cells. Id1-2 are localized in the transformed cell cytoplasm.

In vitro characterization of Id expression in pancreatic cancer cell lines (PANC-1, MIA-PaCa-2, ASPC-1, CAPAN-1, COLO-357) also reveals an overexpression of Id1-2-3 mRNA as well as protein [78,79]. Consistent with this, inhibition of Id2 expression with Id2 antisense RNA results in inhibition of the growth of the PANC-1 human pancreatic cancer cell line [78]. Moreover, in the induced differentiation state PANC-1 exhibits decreased Id2 expression, whereas Id2 level increases in serum-treated PANC-1 cells, leading to proliferation. However, treatment with Id2 sense RNA had no effect on cell proliferation [78]. Finally, overexpression of Id1-2 and, to a lesser extent, Id3 seems to be associated with enhanced proliferative activity of pancreatic cells undergoing tumorigenesis, and inhibition of (re-)differentiation in the case of Id2.

Id1-2-3 may therefore represent markers of pancreatic malignancy [78]. The large variety of bHLH proteins deregulated through overexpression of Ids, principally Id2, may play a key function in the regulation of pancreatic cancer, in addition to being directly connected with pancreatic dysfunction. Further investigation of the downstream genes under Id regulation or potential dysregulation, and the upstream pathway regulating Id expression and function, should bring new insights into the roles of Ids in pancreatic cell proliferation, differentiation, and tumorigenesis.

Liver

An in vivo model based on partial rat hepatectomy and an in vitro model of hepatocyte culture under stimulating factors have been used to determine the Id1 pattern of expression in hepatocytes [80]. Id1 is progressively downregulated during liver development. In terminally differentiated hepatocytes, no Id1 transcripts are detected. However, strong Id1 expression is observed during hepatocyte activation and proliferation, specifically in the mid-late G1 phase of the hepatocyte cell cycle. Conversely, recent data [81]

suggest that Id1 mRNA level diminishes during the dramatic phenotype change occurring through the activation of hepatic stellate cells leading to fibrosis. Analysis of E-box DNA binding activity of nuclear extracts from primary human and rat stellate cells undergoing this activation demonstrates a specific modulation of activity. Since cellular Id1 is downregulated, some bHLH proteins (including MyoD), usually under negative regulation by Id1 in quiescent stellate cells, would be released, thus modifying the global cellular balance of HLH protein, and therefore regulating downstream target genes. This scheme provides insight into the potential role of Id1 during liver-cell differentiation and proliferation, and correlatively cell cycle regulation.

A novel dominant inhibitory HLH protein structurally and functionally related to the Id protein family is implicated in liver-specific gene expression [82]. This new HLH protein, named HHM, for Human Homologue of Maid [83], has a leucine zipper motif and is larger than conventional Id proteins. HHM is a potential partner of E12 proteins during human liver development and transiently during hepatic stem cell activation. HHM inhibits the expression of hepatocyte nuclear factor-4 (HNF-4), a liver-specific gene the promoter of which contains E-boxes as well as, for example, HNF-1α/3α/3β/4/6 [82]. HHM is expressed in fetal but not in adult liver, and during liver regeneration, suggesting a potential role in differentiation and growth regulation in the liver in the adult. HHM may play a role in liver plasticity and perhaps in the tumorigenic process, in a specific hepatic-gene regulation manner.

Thyroid

The thyroid is a ductless endocrine gland, with an epithelial structure secreting hormones directly into blood and extracellular fluid. Stimulation of thyroid cell growth in vitro induces a striking upregulation of Id1 [84]. Conversely, the in vitro induction of cell redifferentiation through the activation of the protein kinase C pathway is associated with a downregulation of Id1 [84]. In contrast to the corresponding normal medullary thyroid phenotype, medullary thyroid cancer (MTC) exhibits characteristics of cell proliferation and loss of cell growth control, dedifferentiation, migration, and invasion, as is typical of most epithelial cancers. MTC is associated with a high level of Id1 expression, as revealed by immunostaining of nucleus and cytoplasm of tumorigenic tissues, as well as mRNA analysis in medullary thyroid cell lines. Id1 is also overexpressed in papillary thyroid cancer and in hyper- and neoplastic thyroid tissues. Interestingly, the high Id1 level of expression is correlated with the RET protooncogene mutation, which leads to constitutive tyrosine kinase activity in some hereditary forms of MTC [84,85]. This suggests that Id1 is a potential mediator of the RET mitogen response. In vitro, differentiation and proliferation of MTC are directly related to the human bHLH achaete-scute homolog-1 protein (Ash-1) [86], a class II bHLH. Ash-1 may be the target of Id1 and could be a thyroid-specific class II bHLH. As in many other tissues, increased Id1 expression in C-cell hyperplasia and MTC could be related to (i) increased levels of functional cell growth stimulatory factors, and/or (ii) decreased presence and activity of growth inhibitory proteins.

Urogenital organs

Kidney and urinary tract

The renal collecting system is composed of glomerular and tubular nephroepithelial cells. During embryogenesis, the kidney undergoes strong morphogenic activities and expresses high levels of Id1-2-3 proteins [11]. Like testis and brain, adult kidney expresses very high levels of Id4 [87,88]. Unlike most tissues, which express two closely related variants, Id1A and Id1B, the mouse adult kidney expresses only Id1A [89]. Thus, variations in either Id1 sequences or of their relative amounts may play a regulatory role.

The bHLH STRA13 [90] as well as the bHLH-PAS proteins HIF-1 [91,92] and potentially ARNT [93,94] are implicated in renal clear cell carcinoma (RCC) resulting from von Hippel-Lindau (VHL) disease, occurring through a VHL tumor suppressor gene mutation. Mouse mesangial cells of the mature glomeruli seem to express both bHLH myf5 and herculin, whereas Ids are no longer expressed [95]. The bHLH Pod-1 is also essential [96] and might be indirectly regulated by Ids. The kidney-specific member of the cadherin superfamily (Ksp-cadherin or cadherin-16) is expressed in the basolateral membrane of renal tubular epithelial cells and possesses within its promoter some E-boxes [97]. Expression of mutated bHLH protein is also implicated in the terminal stages of the very aggressive papillary renal cell carcinoma. Finally, as in other epithelial tissues, the proximal tubes of the kidney are under BMP(-7) and TGF-β regulation [98], suggesting another potential pathway involving Id control of expression.

Prostate and testis

Adult prostatic epithelium consists of basal, secretory, and neuorendocrine cells. Basal cells are situated between the basal membrane and the overlapping secretory cells. The luminal layer consists of tall columnar cells that express the androgen receptor. Prostate cancer development is associated with hormonal imbalance and correlated with advancing age [99]. Induced hormone imbalance by a combination of testosterone and estradiol in the Noble rat model induces a high incidence of prostate hyperplasia, dysplasia, and adenocarcinoma [100]. In these in vivo experimental conditions, Id1 was found at high levels only in prostate tumor cells, whereas premalignant prostate lesions expressed relatively low levels of Id1 [100]. Id1 was also highly over-

expressed in the more poorly differentiated cells, and Id1 levels of expression have been correlated with the histopathological grade of rat tumors [100]. Similar results have been reported for human prostate cancer specimens [101]. Thus, Id1 upregulation might be a biomarker and a mediator of sex hormone-dependent prostate cancer.

Ling et al. [102] also correlated suppression of TGF-β1 levels with in vitro Id1 expression by using their own HPr-1-immortalized prostate epithelial cell line. The TGF-β pathway has been implicated in Id1 transcriptional regulation within epithelial tissues, through TGFβ-R1, swads, and APK [103–105]. Id1 downregulation was associated with induced growth arrest and differentiation. Another study suggested that the $p16^{INK4a}$/pRb pathway might be a downstream target of Id1, potentially increasing the proliferation of LNCaP cells and prostate cancer cells [106]. The transcriptional repression of p16/ink4a by Id1 through Eprt- and Ets-mediated regulation has already been established [107]. Interestingly, overexpression of MMP-7 and clusterin (or TRPM-2) has also been reported during sex hormone-induced prostatic cell tumorigenesis in the Noble rat model [100]. The clusterin gene has also been directly linked to Id-1 in mammary gland (see below). Id1 overexpression is observed in the tumoral stage but not premalignant stages, whereas MMP-7 and clusterin are detectable until formation of dysplastic lesions/cells, which is in good accordance with previous findings [100,108,109]. In contrast, though, significant reduction of Id1 mRNA levels was observed in prostate cancer from human patients in comparison with normal prostate tissues [110,111]. This study [110], as well as that in the previously quoted report [100], was based on the use of cDNA array technique. The discrepant results in Id1 variation might be accounted for by differences in tissue storage, sampling, dissection, and microselection, as well as the prostate zone from which RNA was extracted.

In the testis, Sertoli cells are critical to embryonic development and maintenance of spermatogenesis in the adult. The main regulator of Sertoli cell functions is gonadotropin FSH (follicle-stimulating hormone). FSH is also a regulator of folliculogenesis in the female. The FSH-signalling pathway mediates its action through the FSH-receptor (FSH-R) and, downstream, through bHLH members such as E2A products and REBα, and Id proteins [112–114]. Sertoli cell-specific genes, including transferrin, steroidogenic factor-1, and FSH-R, are under control of an E-box [115–117], and a newly described specific bHLH SERZ is expressed in Sertoli cells [118]. Id proteins themselves are under regulation by FSH in Sertoli cells. The four Ids do not appear to have redundant functions [113,114], and each presents a unique temporal and spatial dynamic pattern of expression within the mouse testis tissues [114]. All are likely to be under hormonal regulation. Id2-3 are abundant in mature nondividing Sertoli cell nuclei and cytoplasm, respectively, whereas Id1 and Id4 are not detectable in these cells [114]. Ids are therefore likely to play an important role in this particular cell lineage.

Endometrium/uterus, cervix, and ovary

A clinical study [119] reported the existence of a relationship between cellular Id1 expression in tissue sections of normal and carcinoma endometria, and patient characteristics. Normal proliferative and secretory endometria were devoid of Id1. Strong Id1 immunoreactivity, indicative of Id1 overexpression, was found in carcinoma tissues. Id1 was present in the nucleus, and sometimes in the cytoplasm, of endometrial carcinoma cells, mostly in cells invading the stroma. Id1 presence and level of expression strongly correlated with the clinical stage, histological grade, cell/tumor aggressiveness and invasiveness (myometrial invasion), and, to a lesser extent, with the clinical outcome of patients. Therefore, Id1 presence could serve as a marker of endometria tumor prognostic [119].

Level of Id1 expression may also be correlated with the prognostic of early-stage cervical cancer and patient survival [120]. Thus, immunohistochemistry of patient cervical tissues demonstrated that Id1 level correlated with clinical outcome, with overexpression of Id1 associated with a higher tumor cell aggressiveness in cervical cancer. Id2 expression, on the other hand, showed a weak negative correlation with cervical cancer prognostic, as in squamous cell carcinomas [26]. The pattern of Id3 expression, while not reaching significance, suggested a trend toward favorable patient prognosis when overexpressed in ovarian tumors. In 70% of ovarian tumors, Id3 was identified as an underexpressed gene, using a cDNA array to compare ovarian cancer cell lines and an immortalized human ovarian surface epithelial cell line [121]. This downregulation was not associated with any Id3 gene mutations nor any loss of heterozygosity at its normal chromosomal localization (1p36.1), though this is a common region of LOH in ovarian cancer. The molecular events driving Id3 reduction of expression in ovarian cancer thus remain to be identified.

Upregulation of Id4, like that of Id1, may be a potential marker of breast and ovarian cancer. Id4 has been identified as an upstream regulator of the *BRCA1* gene (Breast Cancer susceptibility gene) in ovarian cancer and breast cancer, using a very elegant promoter-driven reporter system [122]. An “inverse genomics” approach based on a randomized ribozyme gene library transduced in cells derived from human ovarian and breast cancer (SK-BR-3, T47-D, and PA-1) demonstrated that Id4 could modulate BRCA1 promoter-driven reporter gene expression and endogenous BRCA1 expression. Consistent with this, expression of Id4 and BRCA1 are inversely related in sporadic ovarian and breast cancer: Id4 is upregulated while BRCA1 is downregulated. Use of Id4 sense or antisense vectors inducing Id4 overexpression or knockdown, respectively, has further confirmed the role of Id4 as a potential crucial factor in ovarian/breast tumorigenesis and invasiveness. Id4 may also be involved in the hormone-dependent regulation of BRCA1 expression, since its expression in MCF-7 cells was estrogen dependent in the opposite way from BRCA1 [122].

Therefore, Id4 appears to be involved in the *BRCA1* regulation pathway occurring in ovarian and breast cancer pathogenesis. The BRCA1 regulation through Id4 may also implicate Myc as a potential important player in the ovarian phenotype [123,124].

Mammary gland

Mammary epithelial cell growth and differentiation

Id1 expression was examined during normal mouse mammary gland development in vivo [125]. The expression of β-casein, a marker of differentiation of the mammary epithelial cells, was inversely correlated with Id1 expression. During the initial stages of mammary gland development, when epithelial cells of the ductal trees proliferate and invade the stroma, Id1 was highly expressed. During midpregnancy, Id1 was downregulated and remained undetectable through much of the lactating period, corresponding to a highly differentiated stage [125]. Correlatively, studies of in vitro cellular differentiation detected a rapid and marked decline of Id1 expression [126]. Indeed, when given lactogenic hormones and extracellular matrix (ECM), murine mammary epithelial cells [127] aggregate, arrest growth, form alveolus-like structures, secrete milk proteins [128], and rapidly downregulate Id1 [126].

Consistent with these studies, the ectopic expression of Id1 into SCp2 cells prevents differentiation and milk protein expression, and stimulates cellular proliferation [126]. This study also showed that Id1 upregulates the Zfp289 gene [129] encoding a cytoplasmic protein that is probably the human homologue of the yeast Gcs-1. Gcs-1 is a GAP (GTPase activating protein) necessary to the transition from stationary to proliferation phase in yeast (reentry in cell cycle), and involved in vesicular trafficking and in the regulation of the actin cytoskeleton network. Zfp289 mRNA expression pattern was closely related to that of Id1 during normal mouse mammary gland development.

The expression of Id1 in vitro and in vivo was also shown to be linked with and to induce either proliferation or apoptosis [125]. Thus, depending on extracellular signals as well as cell-cell interactions, Id1 potentially plays dual but opposite functions in mammary gland. Moreover, Id1 downregulates the clusterin gene. This glycoprotein regulates cell-cell interactions [129] and is also involved in apoptosis regulation. The ectopic expression of Id1 in SCp2 cells stimulates cell growth, eventually activating cell migration, and also leads to Id1-related metalloproteinase expression [130]. Under these conditions, a 120-kDa gelatinase is secreted by SCp2-Id1 cells. In nonconfluent SCp2 cells, constitutive expression of Id1 enhances glucocorticoid stimulation of tight junction sealing [131], targeting the adhesive and permeability properties of mammary epithelial cells. Id1 thus helps establish a competent state for mammary epithelial cells to form normal cell-cell contacts [131].

Id-2 also plays an important role in the mammary gland. Id2-deficient female mice display severe lactation defects [132], which could be indicative of a positive role of Id2 during the differentiation process. The functional activity of Stat5 is decreased in Id2−/− mammary glands, implying the participation of Id2 in this pathway [132]. In any case, unlike Id1, Id2 appears in vivo to be associated with mammary epithelial cell differentiation during mammary gland development [125]. Under differentiation conditions, Id2 proteins are detected only when milk proteins start to be produced, and increase throughout late pregnancy and the lactating period [125]. This is confirmed in vitro with the SCp2 cell culture model. Ectopic expression of Id2 in SCp2 cells accelerates differentiation, while Id2 depletion inhibits differentiation [125]. Id2 may therefore be an activator of mammary gland differentiation rather than an inhibitor. Hence, it has been hypothesized that Id1 and Id2 are involved in regulating mammary epithelial cell phenotypes, but by playing opposite roles [125].

Mammary gland tumorigenesis

Unregulated Id1 expression is probably an important event in carcinogenesis of breast, at least for the late events of mammary gland tumorigenesis, that is to say cell migration, invasiveness, and metastasis. In breast cancer biopsies, Id1 protein is more frequently highly expressed in infiltrating carcinomas compared with ductal carcinomas in situ [133]. Therefore, Id1 may serve as a marker of aggressiveness of breast tumors [133]. Id1 mediates the effects of sex steroid hormones on T47D cells, particularly on hormonal control of cell proliferation. Estrogen (E2) stimulates Id1 expression and activates cell proliferation. Progesterone (Pg) represses Id1 expression and inhibits cell growth. Indeed Id1 repression may be an early downstream event, eventually leading to the G1 growth arrest, caused by Pg. Inappropriate Id1 expression may be responsible for the lack of steroid responsiveness shown by some breast cancers.

High levels of Id1 expression also strongly correlate with levels of aggressiveness and invasiveness of several breast cancer lines [130,133]. Some highly aggressive human breast cancer cells lose serum regulation of Id1 expression [133]. This deregulation might be a major cause of the development of a metastatic phenotype in breast cancer cells. Further insight into the role of the Id1 gene in human metastatic breast cancer cells was obtained from analysis of two main regulatory regions within the Id1 promoter [134]. The first, 1.2 kb upstream from the transcription start, consists of Egr-1, YY-1, and CREB/ATF consensus sequences, which are responsible for the serum responsiveness and upregulation of Id1 [134,135]. The second, 200 bp upstream from the transcription start, is a 31-bp sequence responsible for the constitutive expression of Id1 in highly aggressive breast cancer cells. The 31-bp sequence contains SP-1 and NF-1 sites. A molecular complex was identified, containing the corepressor HDAC-1 (condensation of the chromatin), Rb, SP-1, and NF-1. Since SP-1 is expressed in nonaggressive as well as metastatic breast cells, NF-1 may be the key

regulator of *Id1* gene repression in aggressive breast cancer through HDAC-1 recruitment. The loss of NF-1 would impair formation of the NF1/Rb/HDAC1 repressor complex, leading to constitutive expression of Id1 [134].

Another system of regulation of Id gene expression in breast cancer may be related to the BMP factors, and thus the TGF-β superfamily of cytokines and receptors, which have a dual role in normal/abnormal cell homeostasis [103,136]. BMP-2 induces sequential changes and overexpression of Id1-2-3 in the MCF-7 cell line [137]. Id1 is possibly a BMP-2-dependent gene [137–139]. BMP-specific response elements in the Id1 promoter have been identified [105,140]. The BMP effect is likely to be mediated by Smad-1/4 through direct binding to different motifs and activating Id1 transcription.

As noted earlier, Id2 appears to play an opposite role from that of Id1 in mammary epithelial cells. Likewise, the Id1 increase occurring during breast carcinogenesis is associated with a decrease of Id2 expression (Y. Itahana, J. Singh, T. Sumida, J.P. Coppe, J.L. Bennington, and P.Y. Desprez, unpublished data). Downregulation of Id2 during tumor formation is also observed in the small intestine epithelium [52] and, with less certainty, the cervix [26], though Id2 upregulation has been observed in carcinogenesis associated with the epidermis [26], colorectal tissue [48,51], and pancreas [78,79]. A characteristic balance between Id1 and Id2 expression may be altered during the tumoral process of the mammary gland, through the regulation of a bHLH network, perhaps comprising ITF2 [125,141].

Finally, Id4 appears to be also involved in breast cancer development as shown by Beger et al. [122]. Id4 is a regulator of BRCA1, which is downregulated in sporadic breast cancer. Id4 expression results in downregulation of BRCA1 expression and anchorage-independent cell growth.

Other epithelia potentially under Id regulation

The pigmented cells composing the epithelium of the skin—which depends on melanocyte homeostasis—as well as the retinal pigment epithelium of the eyes, may potentially be under Id regulation, either (i) directly, through, e.g., Atonal [142] and Pax-6 [143], or (ii) indirectly, e.g., through Mitf [144,145]. An indirect Id regulation of the corneal epithelium of the eye through IPAS (inhibitory Per/Arnt/Sim domain protein) might also be expected [146]. Another complex function of the skin is its association with hair follicule generation. Id may be involved in this other differentiation process [147]. Similarly, the sensory epithelia of the vestibular and auditory system, composed of inner hair cells and neuroepithelial cells, are dependent on the role of BETA2/NeuroD [148]. This bHLH protein class II as well as Hes are also expressed during mouse olfactory epithelium differentiation [149,150]. Future investigations might focus on determining the role of Ids in olfactory and auditory epithelium-cell fate control. Mist1, a bHLH class II, has been shown to be expressed in the adult epithelium of the lung, stomach, liver, and spleen [151,152]. However, there have been no reports regarding a potential role of Ids or interaction of other factors with Ids in growth and proliferation of some of these tissues. The class B bHLH protein Pod-1/capsulin/epicardin has been implicated in differentiation of spleen tissues [153], and might be indirectly regulated by Ids through sequestration of its broadly expressed partner, E12.

Final perspectives

Since the discovery of Id proteins 12 years ago, the diversity of biological processes they are known to help orchestrate has been enormously expanded. They are now established as important factors for the regulation of cell growth, apoptosis, senescence, cell survival, oncogenesis, tumor progression, and malignancy. In this review, we have highlighted the evidence implicating Id proteins in regulation of epithelia phenotypes. We have taken an organ-by-organ approach to this discussion, but we could have taken a more physiological or molecular one, in which we focused on the metabolic pathways in which Ids are involved. Whatever the approach, these studies make it clear that Ids are a key factor in determining epithelial cell fate, both in normal and pathlogical tissue. Id1, in particular, is overexpressed in almost all epithelial cancers, including epidermis [25,26], esophageal squamous cells [46], small and colorectal intestine [48,51,53], pancreas [78,79,154], thyroid [84], endometrium and cervix [119,120], breast [133], and prostate [100,110] (see Table 2).

Ids may be regulated in epithelial cells through their sensitivity to hormones and growth factors, or because of their involvement in pathways dependent on the extracellular matrix as well as cell-cell interactions. An emerging hypothesis of cancer, for example, involves Id1 dysregulation within hormonal dependent tissues, such as breast, ovary, cervix, endometrium, and prostate. It has also been suggested that different Ids may play opposite roles in a few specific tissues (e.g., Id1 and Id2 in breast), while the same Id may play opposite roles in different tissues (e.g., Id2 in pancreas and breast). These variations may be related to epithelium function or to specific molecular mechanisms associated with Id cellular functions. Finally, studying the roles of Id3 and Id4 in epithelial tissues may lead to new insights in the regulation of epithelial phenotype, perhaps through redundant/overlapping activity able to rescue Id1 or/and Id2 dysregulation, or through completely different effects.

Much work remains to establish how Ids play their key roles in various complex regulation networks, and mediate cellular, environmental, and internal signals. The upstream processes and factors regulating expression of Id genes, as well as the downstream effects of these genes are far from

being completely worked out. Further studies are necessary to elucidate the possible role of Id genes in cell/matrix interactions, as, for example, during epithelial-mesenchymal transition [155,156]. The mechanisms regulating stability, degradation and segregation of Id proteins are also important areas of investigation that could lead to breakthroughs in cancer therapy. However, a major problem slowing down progress in the field of Id HLH biology is the difficulty in obtaining reliable and specific antibodies for each of the four Ids, especially for in vivo detection through immunohistochemistry.

Since most tumors are associated with overexpression of Ids, these proteins are potentially the basis for new diagnostic as well as therapeutic approaches to cancer. As described above, some studies suggest that the aggressiveness of certain tumors is correlated with the level of Id expression. Certain small peptides, molecules, or organic compounds may be useful in inhibiting some specific protein-protein interactions that occur during Id dysregulation and that lead to epithelial cell pathogenicity. Another important avenue of approach involves regulating synthesis of Ids, through antisense technology as well as the molecules promoting Id degradation.

Finally, epithelial tissues constitute valuable tools in the understanding of how Ids function at the molecular level in the overall processes of development, differentiation, and carcinogenesis. Future in vitro and in vivo studies will most certainly bring important new information about the roles of Id proteins in molecular and cell physiology.

Acknowledgments

P.Y. Desprez is supported by grants from the University of California Breast Cancer Research Program (7WB-0026) and from the National Institutes of Health-National Cancer Institute (RO1 CA82548).

References

[1] C. Murre, P.S. McCaw, D. Baltimore, A new DNA binding and dimerization motif in immunoglobulin enhancer binding, daughterless, MyoD, and myc proteins, Cell 56 (1989) 777–783.
[2] Y. Yokota, Id and development, Oncogene 20 (2001) 8290–8298.
[3] M.E. Massari, C. Murre, Helix-loop-helix proteins: regulators of transcription in eucaryotic organisms, Mol. Cell. Biol. 20 (2000) 429–440.
[4] S. Campuzano, Emc, a negative HLH regulator with multiple functions in Drosophila development, Oncogene 20 (2001) B299–B307.
[5] W.R. Atchley, W.M. Fitch, A natural classification of the basic helix-loop-helix class of transcription factors, Proc. Natl. Acad. Sci. USA 94 (1997) 5172–5176.
[6] P.J. Andres-Barquin, M.C. Hernandez, M.A. Israel, Id genes in nervous system development, Histol. Histopathol. 15 (2000) 603–618.
[7] R. Benezra, S. Rafii, D. Lyden, The Id proteins and angiogenesis, Oncogene, 20 (2001) 8334–8341.
[8] R. Benezra, The Id proteins: targets for inhibiting tumor cells and their blood supply, Biochim. Biophys. Acta 1551 (2001) F39–F47.
[9] I. Engel, C. Murre, The function of E- and Id proteins in lymphocyte development, Nat. Rev. Immunol. 1 (2001) 193–199.
[10] R. Benezra, Role of Id proteins in eye and tumor angiogenesis, Trends Cardiovasc. Med. 11 (2001) 232–241.
[11] Y. Jen, K. Manova, R. Benezra, Expression patterns of Id1, Id2, and Id3 are highly related but distinct from that of Id4 during mouse embryogenesis, Dev. Dyn. 207 (1996) 235–252.
[12] D. Lyden, A.Z. Young, D. Zagzag, W. Yan, W. Gerald, R. O'Reilly, B.L. Bader, R.O. Hynes, Y. Zhuang, K. Manova, R. Benezra, Id1 and Id3 are required for neurogenesis, angiogenesis and vascularization of tumour xenografts, Nature 401 (1999) 670–677.
[13] J.D. Norton, R.W. Deed, G. Craggs, F. Sablitzky, Id helix-loop-helix proteins in cell growth and differentiation, Trends Cell. Biol. 8 (1998) 58–65.
[14] J.D. Norton, ID helix-loop-helix proteins in cell growth, differentiation and tumorigenesis, J. Cell Sci. 113 Pt 22 (2000) 3897–3905.
[15] R. Rivera, C. Murre, The regulation and function of the Id proteins in lymphocyte development, Oncogene 20 (2001) 8308–8316.
[16] Z. Zebedee, E. Hara, Id proteins in cell cycle control and cellular senescence, Oncogene 20 (2001) 8317–8325.
[17] Y. Yokota, S. Mori, Role of Id family proteins in growth control, J. Cell Physiol. 190 (2002) 21–28.
[18] C. Murre, G. Bain, M.A. van Dijk, I. Engel, B.A. Furnari, M.E. Massari, J.R. Matthews, M.W. Quong, R.R. Rivera, M.H. Stuiver, Structure and function of helix-loop-helix proteins, Biochim. Biophys. Acta 121B (1994) 129–135.
[19] A. Ephrussi, G.M. Church, S. Tonegawa, W. Gilbert, B lineage—specific interactions of an immunoglobulin enhancer with cellular factors in vivo, Science 227 (1985) 134–140.
[20] R. Benezra, R.L. Davis, D. Lockshon, D.L. Turner, H. Weintraub, The protein Id: a negative regulator of helix-loop-helix DNA binding proteins, Cell 61 (1990) 49–59.
[21] H.M. Ellis, D.R. Spann, J.W. Posakony, extramacrochaetae, a negative regulator of sensory organ development in Drosophila, defines a new class of helix-loop-helix proteins, Cell 61 (1990) 27–38.
[22] M.A. Israel, M.C. Hernandez, M. Florio, P.J. Andres-Barquin, A. Mantani, J.H. Carter, C.M. Julin, Id gene expression as a key mediator of tumor cell biology, Cancer Res. 59 (1999) 1726S–1730S.
[23] A. Lasorella, T. Uo, A. Iavarone, Id proteins at the cross-road of development and cancer, Oncogene 20, (2001) 8326–8333.
[24] F.M. Watt, Stem cell fate and patterning in mammalian epidermis, Curr. Opin. Genet. Dev. 11 (2001) 410–417.
[25] B.M. Schaefer, J. Koch, A. Wirzbach, M.D. Kramer, Expression of the helix-loop-helix protein ID1 in keratinocytes is upregulated by loss of cell-matrix contact, Exp. Cell Res. 266 (2001) 250–259.
[26] K. Langlands, G.A. Down, T. Kealey, Id proteins are dynamically expressed in normal epidermis and dysregulated in squamous cell carcinoma, Cancer Res. 60 (2000) 5929–5933.
[27] R.M. Alani, J. Hasskarl, M. Grace, M.C. Hernandez, M.A. Israel, K. Munger, Immortalization of primary human keratinocytes by the helix-loop-helix protein, Id-1, Proc. Natl. Acad. Sci. USA 96 (1999) 9637–9641.
[28] A.G. Bodnar, M. Ouellette, M. Frolkis, S.E. Holt, C.P. Chiu, G.B. Morin, C.B. Harley, J.W. Shay, S. Lichtsteiner, W.E. Wright, Extension of life-span by introduction of telomerase into normal human cells, Science 279 (1998) 349–352.
[29] B.J. Nickoloff, V. Chaturvedi, P. Bacon, J.Z. Qin, M.F. Denning, M.O. Diaz, Id-1 delays senescence but does not immortalize keratinocytes, J. Biol. Chem. 275 (2000) 27501–27504.
[30] K. Gradin, M.L. Whitelaw, R. Toftgard, L. Poellinger, A. Berghard, A tyrosine kinase-dependent pathway regulates ligand-dependent activation of the dioxin receptor in human keratinocytes, J. Biol. Chem. 269 (1994) 23800–23807.

[31] S. Prabhu, A. Ignatova, S.T. Park, X.H. Sun, Regulation of the expression of cyclin-dependent kinase inhibitor p21 by E2A and Id proteins, Mol. Cell. Biol. 17 (1997) 5888–5896.
[32] W.S. el-Deiry, T. Tokino, V.E. Velculescu, D.B. Levy, R. Parsons, J.M. Trent, D. Lin, W.E. Mercer, K.W. Kinzler, B. Vogelstein, WAF1, a potential mediator of p53 tumor suppression, Cell 75 (1993) 817–825.
[33] M.E. Matsumura, D.R. Lobe, C.A. McNamara, Contribution of the helix-loop-helix factor Id2 to regulation of vascular smooth muscle cell proliferation, J. Biol. Chem. 277 (2002) 7293–7297.
[34] V.I. Sementchenko, D.K. Watson, Ets target genes: past, present and future, Oncogene 19 (2000) 6533–6548.
[35] R. Li, H. Pei, D.K. Watson, Regulation of Ets function by protein-protein interactions, Oncogene 19 (2000) 6514–6523.
[36] M. Prisco, F. Peruzzi, B. Belletti, R. Baserga, Regulation of Id gene expression by type I insulin-like growth factor: roles of Stat3 and the tyrosine 950 residue of the receptor, Mol. Cell. Biol. 21 (2001) 5447–5458.
[37] M. Navarro, B. Valentinis, B. Belletti, G. Romano, K. Reiss, R. Baserga, Regulation of Id2 gene expression by the type 1 IGF receptor and the insulin receptor substrate-1, Endocrinology 142 (2001) 5149–5157.
[38] B.A. Christy, L.K. Sanders, L.F. Lau, N.G. Copeland, N.A. Jenkins, D. Nathans, An Id-related helix-loop-helix protein encoded by a growth factor-inducible gene, Proc. Natl. Acad. Sci. USA 88 (1991) 1815–1819.
[39] M.V. Barone, R. Pepperkok, F.A. Peverali, L. Philipson, Id proteins control growth induction in mammalian cells, Proc. Natl. Acad. Sci. USA 91 (1994) 4985–4988.
[40] E. Hara, T. Yamaguchi, H. Nojima, T. Ide, J. Campisi, H. Okayama, K. Oda, Id-related genes encoding helix-loop-helix proteins are required for G1 progression and are repressed in senescent human fibroblasts, J. Biol. Chem. 269 (1994) 2139–2145.
[41] B. Belletti, R. Drakas, A. Morrione, X. Tu, M. Prisco, T. Yuan, I. Casaburi, R. Baserga, Regulation of Id1 protein expression in mouse embryo fibroblasts by the type 1 insulin-like growth factor receptor, Exp. Cell Res. 277 (2002) 107–118.
[42] J. Liu, W. Shi, D. Warburton, A cysteine residue in the helix-loop-helix domain of Id2 is critical for homodimerization and function, Biochem. Biophys. Res. Commun. 273 (2000) 1042–1047.
[43] P. Minoo, G. Su, H. Drum, P. Bringas, S. Kimura, Defects in tracheoesophageal and lung morphogenesis in Nkx2.1(−/−) mouse embryos, Dev. Biol. 209 (1999) 60–71.
[44] W. Zhu, J. Dahmen, A. Bulfone, M. Rigolet, M.C. Hernandez, W.L. Kuo, L. Puelles, J.L. Rubenstein, M.A. Israel, Id gene expression during development and molecular cloning of the human Id-1 gene, Brain Res. 30 (1995) 312–326.
[45] C.A. Squier, M.J. Kremer, Biology of oral mucosa and esophagus, J. Natl. Cancer Inst. Monogr. 29 (2001) 7–15.
[46] Y.C. Hu, K.Y. Lam, S. Law, J. Wong, G. Srivastava, Identification of differentially expressed genes in esophageal squamous cell carcinoma (ESCC) by cDNA expression array: overexpression of Fra-1, Neogenin, Id-1, and CDC25B genes in ESCC, Clin. Cancer Res. 7 (2001) 2213–2221.
[47] Y. Kimura, H. Shiozaki, Y. Doki, M. Yamamoto, T. Utsunomiya, K. Kawanishi, N. Fukuchi, M. Inoue, T. Tsujinaka, M. Monden, Cytoplasmic beta-catenin in esophageal cancers, Int. J. Cancer 20 (1999) 174–178.
[48] S.P. Rockman, S.A. Currie, M. Ciavarella, E. Vincan, C. Dow, R.J. Thomas, W.A. Phillips, Id2 is a target of the beta-catenin/T cell factor pathway in colon carcinoma, J. Biol. Chem. 276 (2001) 45113–45119.
[49] H. Takeuchi, S. Ozawa, N. Ando, C.H. Shih, K. Koyanagi, M. Ueda, M. Kitajima, Altered p16/MTS1/CDKN2 and cyclin D1/PRAD-1 gene expression is associated with the prognosis of squamous cell carcinoma of the esophagus, Clin. Cancer Res. 3 (12 Pt 1) (1997) 2229–2236.
[50] K. Yoneda, T. Yokoyama, T. Yamamoto, T. Hatabe, T. Osaki, p53 gene mutations and p21 protein expression induced independently of p53, by TGF-beta and gamma-rays in squamous cell carcinoma cells, Eur. J. Cancer 35 (1999) 278–283.
[51] J.W. Wilson, R.W. Deed, T. Inoue, M. Balzi, A. Becciolini, P. Faraoni, C.S. Potten, J.D. Norton, Expression of Id helix-loop-helix proteins in colorectal adenocarcinoma correlates with p53 expression and mitotic index, Cancer Res. 61 (2001) 8803–8810.
[52] B.M. Wice, J.I. Gordon, Forced expression of Id-1 in the adult mouse small intestinal epithelium is associated with development of adenomas, J. Biol. Chem. 273 (1998) 25310–25319.
[53] W. Yan, A.Z. Young, V.C. Soares, R. Kelley, R. Benezra, Y. Zhuang, High incidence of T-cell tumors in E2A-null mice and E2A/Id1 double-knockout mice, Mol. Cell. Biol. 17 (1997) 7317–7327.
[54] A.R. Moser, H.C. Pitot, W.F. Dove, A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse, Science 247 (1990) 322–324.
[55] G. Rindi, C. Ratineau, A. Ronco, M.E. Candusso, M. Tsai, A.B. Leiter, Targeted ablation of secretin-producing cells in transgenic mice reveals a common differentiation pathway with multiple enteroendocrine cell lineages in the small intestine, Development 126 (1999) 4149–4156.
[56] C. Ratineau, M.W. Petry, H. Mutoh, A.B. Leiter, Cyclin D1 represses the basic helix-loop-helix transcription factor, BETA2/NeuroD, J. Biol. Chem. 277 (2002) 8847–8853.
[57] Q. Yang, N.A. Bermingham, M.J. Finegold, H.Y. Zoghbi, Requirement of Math1 for secretory cell lineage commitment in the mouse intestine, Science 294 (2001) 2155–2158.
[58] A. Lasorella, M. Noseda, M. Beyna, Y. Yokota, A. Iavarone, Id2 is a retinoblastoma protein target and mediates signalling by Myc oncoproteins, Nature 407 (2000) 592–598.
[59] A. Lasorella, R. Boldrini, C. Dominici, A. Donfrancesco, Y. Yokota, A. Inserra, A. Iavarone, Id2 is critical for cellular proliferation and is the oncogenic effector of N-myc in human neuroblastoma, Cancer Res. 62 (2002) 301–306.
[60] J. Behrens, Control of beta-catenin signaling in tumor development, Ann. NY Acad. Sci. 10 (2000) 21–33.
[61] T.C. He, A.B. Sparks, C. Rago, H. Hermeking, L. Zawel, L.T. da Costa, P.J. Morin, B. Vogelstein, K.W. Kinzler, Identification of c-MYC as a target of the APC pathway, Science 281 (1998) 1509–1512.
[62] A. Iavarone, P. Garg, A. Lasorella, J. Hsu, M.A. Israel, The helix-loop-helix protein Id-2 enhances cell proliferation and binds to the retinoblastoma protein, Genes Dev. 8 (1994) 1270–1284.
[63] A. Lasorella, A. Iavarone, M.A. Israel, Id2 specifically alters regulation of the cell cycle by tumor suppressor proteins, Mol. Cell. Biol. 16 (1996) 2570–2578.
[64] A. Pagliuca, P. Gallo, P. De Luca, L. Lania, Class A helix-loop-helix proteins are positive regulators of several cyclin-dependent kinase inhibitors' promoter activity and negatively affect cell growth, Cancer Res. 60 (2000) 1376–1382.
[65] J. Jensen, P. Serup, C. Karlsen, T.F. Nielsen, O.D. Madsen, mRNA profiling of rat islet tumors reveals nkx 6.1 as a beta-cell-specific homeodomain transcription factor, J. Biol. Chem. 271 (1996) 18749–18758.
[66] S.R. Cordle, J. Whelan, E. Henderson, H. Masuoka, P.A. Weil, R. Stein, Insulin gene expression in nonexpressing cells appears to be regulated by multiple distinct negative-acting control elements, Mol. Cell. Biol. 11 (1991) 2881–2886.
[67] G.L. Robinson, S.R. Cordle, E. Henderson, P.A. Weil, G. Teitelman, R. Stein, Isolation and characterization of a novel transcription factor that binds to and activates insulin control element-mediated expression, Mol. Cell. Biol. 14 (1994) 6704–6714.
[68] Y. Qiu, M. Guo, S. Huang, R. Stein, Insulin gene transcription is mediated by interactions between the p300 coactivator and PDX-1, BETA2, and E47, Mol. Cell. Biol. 22 (2002) 412–420.

[69] J.C. Lee, S.B. Smith, H. Watada, J. Lin, D. Scheel, J. Wang, R.G. Mirmira, M.S. German, Regulation of the pancreatic pro-endocrine gene neurogenin 3, Diabetes 50 (2001) 928–936.
[70] G. Gradwohl, A. Dierich, M. LeMeur, F. Guillemot, Neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas, Proc. Natl. Acad. Sci. USA 97 (2000) 1607–1611.
[71] J. Jensen, R.S. Heller, T. Funder-Nielsen, E.E. Pedersen, C. Lindsell, G. Weinmaster, O.D. Madsen, P. Serup, Independent development of pancreatic alpha- and beta-cells from neurogenin3-expressing precursors: a role for the notch pathway in repression of premature differentiation, Diabetes 49 (2000) 163–176.
[72] J. Jensen, E.E. Pedersen, P. Galante, J. Hald, R.S. Heller, M. Ishibashi, R. Kageyama, F. Guillemot, P. Serup, O.D. Madsen, Control of endodermal endocrine development by Hes-1, Nat. Genet. 24 (2000) 36–44.
[73] A. Krapp, M. Knofler, B. Ledermann, K. Burki, C. Berney, N. Zoerkler, O. Hagenbuchle, P.K. Wellauer, The bHLH protein PTF1-p48 is essential for the formation of the exocrine and the correct spatial organization of the endocrine pancreas, Genes Dev. 12 (1998) 3752–3763.
[74] S.D. Rose, G.H. Swift, M.J. Peyton, R.E. Hammer, R.J. MacDonald, The role of PTF1-P48 in pancreatic acinar gene expression, J. Biol. Chem. 276 (2001) 44018–44026.
[75] A.V. Biankin, J.G. Kench, A.L. Morey, C.S. Lee, S.A. Biankin, D.R. Head, T.B. Hugh, S.M. Henshall, R.L. Sutherland, Overexpression of p21 (WAF1/CIP1) is an early event in the development of pancreatic intraepithelial neoplasia, Cancer Res. 61 (2001) 8830–8837.
[76] F.A. Peverali, T. Ramqvist, R. Saffrich, R. Pepperkok, M.V. Barone, L. Philipson, Regulation of G1 progression by E2A and Id helix-loop-helix proteins, EMBO J. 13 (1994) 4291–4301.
[77] A.L. Warshaw, C. Fernandez-del Castillo, Pancreatic carcinoma, N. Engl. J. Med. 326 (1992) 455–465.
[78] F.J. Kleef, T. Ishiwata, H. Friess, M.W. Buchler, M.A. Israel, M. Korc, The helix-loop-helix protein Id2 is overexpressed in human pancreatic cancer, Cancer Res. 58 (1998) 3769–3772.
[79] H. Maruyama, J. Kleeff, S. Wildi, H. Friess, M.W. Buchler, M.A. Israel, M. Korc, Id-1 and Id-2 are overexpressed in pancreatic cancer and in dysplastic lesions in chronic pancreatitis, Am. J. Pathol. 155 (1999) 815–822.
[80] C. Le Jossic, G.P. Ilyin, P. Loyer, D. Glaise, S. Cariou, C. Guguen-Guillouzo, Expression of helix-loop-helix factor Id-1 is dependent on the hepatocyte proliferation and differentiation status in rat liver and in primary culture, Cancer Res. 54 (1994) 6065–6068.
[81] K.J. Vincent, E. Jones, M.J. Arthur, D.E. Smart, J. Trim, M.C. Wright, D.A. Mann, Regulation of E-box DNA binding during in vivo and in vitro activation of rat and human hepatic stellate cells, Gut 49 (2001) 713–719.
[82] S. Terai, H. Aoki, K. Ashida, S.S. Thorgeirsson, Human homologue of maid: A dominant inhibitory helix-loop-helix protein associated with liver-specific gene expression, Hepatology 32 (2000) 357–366.
[83] S.Y. Hwang, B. Oh, A. Fuchtbauer, E.M. Fuchtbauer, K.R. Johnson, D. Solter, B.B. Knowles, Maid: a maternally transcribed novel gene encoding a potential negative regulator of bHLH proteins in the mouse egg and zygote, Dev. Dyn. 209 (1997) 217–226.
[84] E. Kebebew, P.A. Treseler, Q.Y. Duh, O.H. Clark, The helix-loop-helix transcription factor, Id-1, is overexpressed in medullary thyroid cancer, Surgery 128 (2000) 952–957.
[85] D.J. Marsh, D.L. Learoyd, B.G. Robinson, Medullary thyroid carcinoma: recent advances and management update, Thyroid 5 (1995) 407–424.
[86] H. Chen, E.B. Carson-Walter, S.B. Baylin, B.D. Nelkin, D.W. Ball, Differentiation of medullary thyroid cancer by C-Raf-1 silences expression of the neural transcription factor human achaete-scute homolog-1, Surgery 120 (1996) 168–172.
[87] V. Riechmann, I. van Cruchten, F. Sablitzky, The expression pattern of Id4, a novel dominant negative helix-loop-helix protein, is distinct from Id1, Id2 and Id3, Nucleic Acids Res. 22 (1994) 749–755.
[88] I. van Cruchten, E. Cinato, M. Fox, E.R. King, J.S. Newton, V. Riechmann, F. Sablitzky, Structure, chromosomal localisation and expression of the murine dominant negative helix-loop-helix Id4 gene, Biochim. Biophys. Acta 1443 (1998) 55–64.
[89] M.C. Hernandez, P.J. Andres-Barquin, M.A. Israel, Molecular cloning of the cDNA encoding a helix-loop-helix protein, mouse ID1B: tissue-specific expression of ID1A and ID1B genes, Biochim. Biophys. Acta 308 (1996) 28–30.
[90] M. Krieg, R. Haas, H. Brauch, T. Acker, I. Flamme, K.H. Plate, Up-regulation of hypoxia-inducible factors HIF-1alpha and HIF-2alpha under normoxic conditions in renal carcinoma cells by von Hippel-Lindau tumor suppressor gene loss of function, Oncogene 19 (2000) 5435–5443.
[91] A.V. Ivanova, S.V. Ivanov, A. Danilkovitch-Miagkova, M.I. Lerman, Regulation of STRA13 by the von Hippel-Lindau tumor suppressor protein, hypoxia, and the UBC9/ubiquitin proteasome degradation pathway, J. Biol. Chem. 276 (2001) 15306–15315.
[92] S. Hara, J. Hamada, C. Kobayashi, Y. Kondo, N. Imura, Expression and characterization of hypoxia-inducible factor (HIF)-3alpha in human kidney: suppression of HIF-mediated gene expression by HIF-3alpha, Biochem. Biophys. Res. Commun. 287 (2001) 808–813.
[93] E. Maltepe, B. Keith, A.M. Arsham, J.R. Brorson, M.C. Simon, The role of ARNT2 in tumor angiogenesis and the neural response to hypoxia, Biochem. Biophys. Res. Commun. 273 (2000) 231–238.
[94] B. Keith, D.M. Adelman, M.C. Simon, Targeted mutation of the murine arylhydrocarbon receptor nuclear translocator 2 (Arnt2) gene reveals partial redundancy with Arnt, Proc. Natl. Acad. Sci. USA 98 (2001) 6692–6697.
[95] T. Imabayashi, N. Iehara, H. Takeoka, M. Uematsu-Yanagita, H. Kataoka, S. Nishikawa, H. Sano, M. Yokode, A. Fukatsu, T. Kita, T. Doi, Expression of basic helix-loop-helix proteins in the glomeruli, Clin. Nephrol. 55 (2001) 53–58.
[96] S.E. Quaggin, L. Schwartz, S. Cui, P. Igarashi, J. Deimling, M. Post, J. Rossant, The basic-helix-loop-helix protein pod1 is critically important for kidney and lung organogenesis, Development 126 (1999) 5771–5783.
[97] D.A. Whyte, C. Li, R.B. Thomson, S.L. Nix, R. Zanjani, S.L. Karp, P.S. Aronson, P. Igarashi, Ksp-cadherin gene promoter. I. Characterization and renal epithelial cell-specific activity, Am. J. Physiol. 277 (1999) F587–F598.
[98] S.E. Gould, M. Day, S.S. Jones, H. Dorai, BMP-7 regulates chemokine, cytokine, and hemodynamic gene expression in proximal tubule cells, Kidney Int. 61 (2002) 51–60.
[99] F. Sciarra, V. Toscano, Role of estrogens in human benign prostatic hyperplasia, Arch. Androl. 44 (2000) 213–220.
[100] X.S. Ouyang, X. Wang, D.T. Lee, S.W. Tsao, Y.C. Wong, Up-regulation of TRPM-2, MMP-7 and ID-1 during sex hormone-induced prostate carcinogenesis in the Noble rat, Carcinogenesis 22 (2001) 965–973.
[101] X.S. Ouyang, X. Wang, D.T. Lee, S.W. Tsao, Y.C. Wong, Over expression of ID-1 in prostate cancer, J. Urol. 167 (2002) 2598–2602.
[102] M.T. Ling, X. Wang, S.W. Tsao, Y.C. Wong, Down-regulation of Id-1 expression is associated with TGFbeta1-induced growth arrest in prostate epithelial cells, Biochim. Biophys. Acta 1570 (2002) 145–152.
[103] M.J. Goumans, G. Valdimarsdottir, S. Itoh, A. Rosendahl, P. Sideras, P. ten Dijke, Balancing the activation state of the endothelium via two distinct TGF-beta type I receptors, EMBO J. 21 (2002) 1743–1753.
[104] A. Hollnagel, V. Oehlmann, J. Heymer, U. Ruther, A. Nordheim, Id genes are direct targets of bone morphogenetic protein induction in embryonic stem cells, J. Biol. Chem. 274 (1999) 19838–19845.

[105] O. Korchynskyi, P. ten Dijke, Identification and functional characterization of distinct critically important bone morphogenetic protein-specific response elements in the Id1 promoter, J. Biol. Chem. 277 (2002) 4883–4891.
[106] X.S. Ouyang, X. Wang, M.T. Ling, H.L. Wong, S.W. Tsao, Y.C. Wong, Id-1 stimulates serum independent prostate cancer cell proliferation through inactivation of p16(INK4a)/pRB pathway, Carcinogenesis 23 (2002) 721–725.
[107] D. Polsky, A.Z. Young, K.J. Busam, R.M. Alani, The transcriptional repressor of p16/Ink4a, Id1, is up-regulated in early melanomas, Cancer Res. 61 (2001) 6008–6011.
[108] J.D. Knox, C. Wolf, K. McDaniel, V. Clark, M. Loriot, G.T. Bowden, R.B. Nagle, Matrilysin expression in human prostate carcinoma, Mol. Carcinog. 15 (1996) 57–63.
[109] J. Steinberg, et al., Intracellular levels of SGP-2 (Clusterin) correlate with tumor grade in prostate cancer, Clin. Cancer Res. 3 (1997) 1707–1711.
[110] A. Chetcuti, S. Margan, S. Mann, P. Russell, D. Handelsman, J. Rogers, Q. Dong, Identification of differentially expressed genes in organ-confined prostate cancer by gene expression array, Prostate 47 (2001) 132–140.
[111] T. Ernst, M. Hergenhahn, M. Kenzelmann, C.D. Cohen, M. Bonrouhi, A. Weninger, R. Klaren, E.F. Grone, M. Wiesel, C. Gudemann, J. Kuster, W. Schott, G. Staehler, M. Kretzler, M. Hollstein, H.J. Grone, Decrease and gain of gene expression are equally discriminatory markers for prostate carcinoma: a gene expression analysis on total and microdissected prostate tissue, Am. J. Pathol. 60 (2002) 2169–2180.
[112] J. Chaudhary, G. Kim, M.K. Skinner, Expression of the basic helix-loop-helix protein REBalpha in rat testicular Sertoli cells, Biol Reprod. 60 (1999) 1244–1250.
[113] J. Chaudhary, R. Mosher, G. Kim, M.K. Skinner, Role of winged helix transcription factor (WIN) in the regulation of Sertoli cell differentiated functions: WIN acts as an early event gene for follicle-stimulating hormone, Endocrinology 141 (2000) 2758–2766.
[114] F. Sablitzky, A. Moore, M. Bromley, R.W. Deed, J.S. Newton, J.D. Norton, Stage- and subcellular-specific expression of Id proteins in male germ and Sertoli cells implicates distinctive regulatory roles for Id proteins during meiosis, spermatogenesis, and Sertoli cell function, Cell Growth Differ. 9 (1998) 1015–1024.
[115] J. Chaudhary, M.K. Skinner, E-box and cyclic adenosine monophosphate response elements are both required for follicle-stimulating hormone-induced transferrin promoter activation in Sertoli cells, Endocrinology 140 (1999) 1262–1271.
[116] M.A. Daggett, D.A. Rice, L.L. Heckert, Expression of steroidogenic factor 1 in the testis requires an E box and CCAAT box in its promoter proximal region, Biol. Reprod. 62 (2000) 670–679.
[117] T.L. Goetz, T.L. Lloyd, M.D. Griswold, Role of E box and initiator region in the expression of the rat follicle-stimulating hormone receptor, J. Biol. Chem. 271 (1996) 33317–33324.
[118] J. Chaudhary, M.K. Skinner, Identification of a novel gene product, Sertoli cell gene with a zinc finger domain, that is important for FSH activation of testicular Sertoli cells, Endocrinology 143 (2002) 426–435.
[119] N. Takai, T. Miyazaki, K. Fujisawa, K. Nasu, I. Miyakawa, Id1 expression is associated with histological grade and invasive behavior in endometrial carcinoma, Cancer Lett. 165 (2001) 185–193.
[120] M. Schindl, G. Oberhuber, A. Obermair, S.F. Schoppman, B. Karner, P. Birner, Overexpression of Id-1 protein is a marker for unfavorable prognosis in early-stage cervical cancer, Cancer Res. 61 (2001) 5703–5706.
[121] J.M. Arnold, M. Cummings, D. Purdie, G. Chenevix-Trench, Reduced expression of intercellular adhesion molecule-1 in ovarian adenocarcinomas, Br. J. Cancer. 85 (2001) 1351–1358.
[122] C. Beger, L.N. Pierce, M. Kruger, E.G. Marcusson, J.M. Robbins, P. Welcsh, P.J. Welch, K. Welte, M.C. King, J.R. Barber, F. Wong-Staal, Identification of Id4 as a regulator of BRCA1 expression by using a ribozyme-library-based inverse genomics approach, Proc. Natl. Acad. Sci. USA 98 (2001) 130–135.
[123] O.W. Prall, E.M. Rogan, E.A. Musgrove, C.K. Watts, R.L. Sutherland, c-Myc or cyclin D1 mimics estrogen effects on cyclin E-Cdk2 activation and cell cycle reentry, Mol. Cell. Biol. 18 (1998) 4499–4508.
[124] Q. Wang, H. Zhang, K. Kajino, M.I. Greene, BRCA1 binds c-Myc and inhibits its transcriptional and transforming activity in cells, Oncogene 17 (1998) 1939–1948.
[125] S. Parrinello, C.Q. Lin, K. Murata, Y. Itahana, J. Singh, A. Krtolica, J. Campisi, P.Y. Desprez, Id-1, ITF-2, and Id-2 comprise a network of helix-loop-helix proteins that regulate mammary epithelial cell proliferation, differentiation, and apoptosis, J. Biol. Chem. 276 (2001) 39213–39219.
[126] P.Y. Desprez, E. Hara, M.J. Bissell, J. Campisi, Suppression of mammary epithelial cell differentiation by the helix-loop-helix protein Id-1, Mol. Cell Biol. 15 (1995) 3398–3404.
[127] C. Schmidhauser, M.J. Bissell, C.A. Myers, G.F. Casperson, Extracellular matrix and hormones transcriptionally regulate bovine beta-casein 5′sequences in stably transfected mouse mammary cells, Proc. Natl. Acad. Sci. USA 87 (1990) 9118–9122.
[128] C.D. Roskelley, P.Y. Desprez, M.J. Bissell, Extracellular matrix-dependent tissue-specific gene expression in mammary epithelial cells requires both physical and biochemical signal transduction, Proc. Natl. Acad. Sci. USA 91 (1994) 12378–12382.
[129] J. Singh, Y. Itahana, S. Parrinello, K. Murata, P.Y. Desprez, Molecular cloning and characterization of a zinc finger protein involved in Id-1-stimulated mammary epithelial cell growth, J. Biol. Chem. 276 (2001) 11852–11858.
[130] P.Y. Desprez, C.Q. Lin, N. Thomasset, C.J. Sympson, M.J. Bissell, J. Campisi, A novel pathway for mammary epithelial cell invasion induced by the helix-loop-helix protein Id-1, Mol. Cell. Biol. 18 (1998) 4577–4588.
[131] P. Woo, A. Cercek, P.Y. Desprez, G.L. Firestone, Involvement of the helix-loop-helix protein Id-1 in the glucocorticoid regulation of tight junctions in mammary epithelial cells, J. Biol. Chem. 275 (2000) 28649–28658.
[132] S. Mori, S.I. Nishikawa, Y. Yokota, Lactation defect in mice lacking the helix-loop-helix inhibitor Id2, EMBO J. 19 (2000) 5772–5781.
[133] C.Q. Lin, J. Singh, K. Murata, Y. Itahana, S. Parrinello, S.H. Liang, C.E. Gillett, J. Campisi, P.Y. Desprez, A role for Id-1 in the aggressive phenotype and steroid hormone response of human breast cancer cells, Cancer Res. 60 (2000) 1332–1340.
[134] J. Singh, K. Murata, Y. Itahana, P.Y. Desprez, Constitutive expression of the Id-1 promoter in human metastatic breast cancer cells is linked with the loss of NF-1/Rb/HDAC-1 transcription repressor complex, Oncogene 21 (2002) 1812–1822.
[135] O. Tournay, R. Benezra, Transcription of the dominant-negative helix-loop-helix protein Id1 is regulated by a protein complex containing the immediate-early response gene Egr-1, Mol. Cell. Biol. 16 (1996) 2418–2430.
[136] R.J. Akhurst, R. Derynck, TGF-beta signaling in cancer—a double-edged sword, Trends Cell. Biol. 11 (2001) S44–S51.
[137] J.H. Clement, N. Marr, A. Meissner, M. Schwalbe, W. Sebald, K.O. Kliche, K. Hoffken, S. Wolfe, Bone morphogenetic protein 2 (BMP-2) induces sequential changes of Id gene expression in the breast cancer cell line MCF-7, J. Cancer Res. Clin. Oncol. 126 (2000) 271–279.
[138] T. Ogata, J.M. Wozney, R. Benezra, M. Noda, Bone morphogenetic protein 2 transiently enhances expression of a gene, Id (inhibitor of differentiation), encoding a helix-loop-helix molecule in osteoblast-like cells, Proc. Natl. Acad. Sci. USA 90 (1993) 9219–9222.
[139] T. Katagiri, A. Yamaguchi, M. Komaki, E. Abe, N. Takahashi, T. Ikeda, V. Rosen, J.M. Wozney, A. Fujisawa-Sehara, T. Suda, Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage, J. Cell Biol. 127 (1994) 1755–1761.

[140] T. Lopez-Rovira, E. Chalaux, J. Massague, J.L. Rosa, F. Ventura, Direct binding of Smad1 and Smad4 to two distinct motifs mediates bone morphogenetic protein-specific transcriptional activation of Id1 gene, J. Biol. Chem. 277 (2002) 3176–3185.
[141] F.T. Kolligs, M.T. Nieman, I. Winer, G. Hu, D. van Mater, Y. Feng, I.M. Smith, R. Wu, Y. Zhai, K.R. Cho, E.R. Fearon, ITF-2, a downstream target of the Wnt/TCF pathway, is activated in human cancers with beta-catenin defects and promotes neoplastic transformation, Cancer Cell. 1 (2002) 145–155.
[142] A. Baonza, T. Casci, M. Freeman, A primary role for the epidermal growth factor receptor in ommatidial spacing in the Drosophila eye, Curr. Biol. 11 (2001) 396–404.
[143] N. Planque, L. Leconte, F.M. Coquelle, P. Martin, S. Saule, Specific Pax-6/microphthalmia transcription factor interactions involve their DNA-binding domains and inhibit transcriptional properties of both proteins, J. Biol. Chem. 276 (2001) 29330–29337.
[144] M. Tachibana, MITF: a stream flowing for pigment cells, Pigment Cells Res. 13 (2000) 230–240.
[145] M.D. Galibert, S. Carreira, C.R. Goding, The Usf-1 transcription factor is a novel target for the stress-responsive p38 kinase and mediates UV-induced tyrosinase expression, EMBO J. 20 (2001) 5022–5031.
[146] Y. Makino, R. Cao, K. Svensson, G. Bertilsson, M. Asman, H. Tanaka, Y. Cao, A. Berkenstam, L. Poellinger, Inhibitory PAS domain protein is a negative regulator of hypoxia-inducible gene expression, Nature 414 (2001) 550–554.
[147] R. Kopan, H. Weintraub, Mouse notch: expression in hair follicles correlates with cell fate determination, J. Cell Biol. 121 (1993) 631–641.
[148] M. Liu, F.A. Pereira, S.D. Price, M.J. Chu, C. Shope, D. Himes, R.A. Eatock, W.E. Brownell, A. Lysakowski, M.J. Tsai, Essential role of BETA2/NeuroD1 in development of the vestibular and auditory systems, Genes Dev. 14 (2000) 2839–2854.
[149] K. Nibu, K. Kondo, Y. Ohta, T. Ishibashi, J.L. Rothstein, K. Kaga, Expression of NeuroD and TrkB in developing and aged mouse olfactory epitheliu, Neuroreport 12 (2001) 1615–1619.
[150] E. Cau, G. Gradwohl, S. Casarosa, R. Kageyama, F. Guillemot, Hes genes regulate sequential stages of neurogenesis in the olfactory epithelium, Development 127 (2000) 2323–2332.
[151] C. Lemercier, R.Q. To, B.J. Swanson, G.E. Lyons, S.F. Konieczny, Mist1: a novel basic helix-loop-helix transcription factor exhibits a developmentally regulated expression pattern, Dev. Biol. 182 (1997) 101–113.
[152] C.L. Pin, A.C. Bonvissuto, S.F. Konieczny, Mist1 expression is a common link among serous exocrine cells exhibiting regulated exocytosis, Anat. Rec. 259 (2000) 157–167.
[153] J. Lu, P. Chang, J.A. Richardson, L. Gan, H. Weiler, E.N. Olson, The basic helix-loop-helix transcription factor capsulin controls spleen organogenesis, Proc. Natl. Acad. Sci. USA 97 (2000) 9525–9530.
[154] B.M. Wice, E. Bernal-Mizrachi, M.A. Permutt, Glucose and other insulin secretagogues induce, rather than inhibit, expression of Id-1 and Id-3 in pancreatic islet beta cells, Diabetologia 44 (2001) 453–463.
[155] M.A. Perez-Moreno, A. Locascio, I. Rodrigo, G. Dhondt, F. Portillo, M.A. Nieto, A. Cano, A new role for E12/E47 in the repression of E-cadherin expression and epithelial-mesenchymal transitions, J. Biol. Chem. 276 (2001) 27424–27431.
[156] Y. Hegerfeldt, M. Tusch, E.B. Brocker, P. Friedl, Collective cell movement in primary melanoma explants: plasticity of cell-cell interaction, beta1-integrin function, and migration strategies, Cancer Res. 62 (2002) 2125–2130.